Training
Identification of Marijuana

I. Introduction:

Marijuana samples will be tested and analyzed by microscopic (morphological) analysis, along with The Modified Duquenois Test, or The Rapid Modified Duquenois Test. Marijuana samples for which the morphology of the plant material cannot be confirmed, e.g. residues, should be analyzed by GC/MS. Marijuana samples exceeding five pounds (results will most likely be required for trial) will, in addition to the Duquenois Test, be analyzed by GC/MS as a confirmation.

II. Reagents:

A.) Modified Duquenois Test:

1. Petroleum Ether
2. Duquenois Reagent: 10 mg Vanillin, 7 mL Acetaldehyde, in 500 mL of Ethanol
3. Concentrated Hydrochloric Acid
4. Chloroform

B.) Rapid Modified Duquenois Test:

1. Duquenois Reagent: 10 mg Vanillan, 7 mL Acetaldehyde, in 500 mL of Ethanol.
2. Concentrated Hydrochloric Acid
3. Chloroform

C.) Chromatography by GC/MS as Confirmation:

1. Petroleum Ether
2. Methylene Chloride/Isopropyl Alcohol (9:1)
3. Methanol: used for solvent rinse on the instrument

III. Equipment:

A.) Analytical Balance
B.) Microscope and slides
C.) Test Tubes
D.) Hot Plate (for when samples are moist)
E.) Porcelain Dish
F.) Hewlett Packard 6890 GC or Hewlett Packard 5890 GC.
G.) 2 mL Autosampler Vials with Teflon caps
H.) 10 mL Volumetric flask

I.) GC/MS: HP 6890/5973 or HP 5890/5972 Series.

IV. Procedure:

A.) Microscopic (Morphology) Test:

1. Open sample bag and remove portion of sample and place on slide.
2. Identify characteristic morphological features of Cannabis sativa by concentrating on leaves, small twigs, seed hulls, cystolith hairs, glandular hairs, and flowering tops.
3. Both cystolith hairs and glandular hairs should be observed to be considered positive.

B.) Modified Duquenois Test:

1. Extract 30-100 mg of sample with 15-20 mL of Petroleum Ether
2. Filter and evaporate the filtrate in a white porcelain dish.
3. Add 2mL of Duquenois reagent and stir to bring residue into solution.
4. Add 2 mL of concentrated Hydrochloric Acid, stir and let stand 10 minutes. A color will develop.
5. Transfer colored solution to labeled test tube and shake with 1-2 mL of Chloroform.
6. If marihuana is present in the sample, the violet color will be transferred to the Chloroform layer.

C.) Rapid Modified Duquenois Test:

1. Place 25-60 mg of dry crushed marijuana in a test tube and shake with 2 mL of Duquenois reagent for 1 minute.
2. Add an equal amount of concentrated Hydrochloric Acid and observe the color changes to a final violet shade.
3. Shake the mixture with 1-2 mL Chloroform.
4. If marihuana is present in the sample the violet shade will be transferred to the Chloroform layer.

D.) Chromatography by GC/MS

1. For residue samples that cannot be identified microscopically, samples will be run on GC/MS for confirmation.
2. Rinse the residue into a beaker using petroleum ether, evaporate in the hood.
3. Dissolve the residue in 1-2 mL Methylene Chloride/Isopropyl Alcohol (9:1), transfer to a GC vial and cap.

4. Place vial on autosampler with the following sequence: standard, blank, samples, standard.

5. For marijuana samples that are 5 lbs. or heavier, samples will be run on GC/MS for confirmation.
6. Add between 30-100 mg of sample to an autosampler vial, enough so the bottom of the vial is covered.
7. Add 1-2 mL of Petroleum Ether to the autosampler vial, cap and shake a few times.
8. Place vial on autosampler with the following sequence: standard, blank, samples, standard.

9. GC/MS conditions:
   Method: EXP
   Oven:
   Initial Temp: 230°C
   Initial Time: 0.00 min.
   Max. Temp: 325°C
   Equilibrium Time: 0.50 min.
   Rate: 10.0°/sec
   Final Temp: 280°C
   Run Time: 10.00 min.
   Inlet:
   Injector Temp: 250°C
   Mode: Split
   Pressure: 31.65 psi
   Gas Type: Helium
   Column:
   Initial Flow: 1.0 mL/min.
   Column: Capillary (30m long, 320 um diameter) HP1MS
10. If THC is present in the sample, the GC/MS will detect the peak for Delta-9-THC, and will generate a report with chromatograph. Delta-9-THC data is stored in the GC/MS library and is used to confirm hits detected on the GC/MS. The library can also be accessed directly without needing to run a sample first. (See graph, last page).

E.) THC Quantitation Procedure:

1. Make Stock Solution: $C_{30}H_{64}$, 2 mg/mL in Petroleum Ether
2. Make Working Solution by transferring 5 mL of stock solution to a 10 mL volumetric flask, add 1 mL THC standard (10 mg/mL in Ethanol), and bring to volume with Petroleum Ether.
3. Prepare sample by measuring 400 mg of sample and soaking in Petroleum Ether overnight. Next, filter and evaporate to about 3 mL (do not let go dry). In a 10 mL volumetric flask, add 5 mL of stock solution, 3 mL of sample, then

rinse container containing the sample with Petroleum Ether to bring volume volumetric flask to volume.

4. Transfer solutions mentioned above to appropriately labeled autosampler vials and run on GC. The sequence run is as follows: THC STD., THC STD (Calib.), THC STD., BLK, SAMPLE (S), THC STD.
5. Calculations for % THC:

$$\frac{\text{PK HT THC(sample)}}{\text{PK HT}(C_{30}H_{64})} \times \frac{\text{PK HT}(C_{30}H_{64}\text{ STD})}{\text{PK HT THC(STD)}} \times \frac{\text{mg THC(STD)}}{\text{mL(STD)}} \times \frac{\text{tot.vol sample(mL)} \times 100\%}{\text{wt.sample (mg)}}$$

V. Results:

A.) A positive result for Marijuana is reported when the morphological examination of the plant material is positive and the Duquenois test is positive. GC/MS retention time and spectra should match standard delta-9-tetrahydrocanabinol when this test is used.

B.) For the Microscopic and Duquenois Tests, record results, date, sample number, in logbook and results, date, chemist initials on sample cards that are with samples.

C.) Sample cards will then be reported to client on formal Certificate of Analysis.

D.) When performing analysis on GC/MS, print out results with chromatographs, reports as stated above. However, file chromatographs so they may be accessed if needed.